

Application No.: 09/586,628

Filing Date: June 5, 2000

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29. (New) In a method of separating charged molecular species, the method comprising causing a charged molecular species to migrate in a separation medium by the influence of an applied electric field, the improvement wherein the separation medium comprises two different polymers that do not phase separate when dissolved in solution and that are entangled to form an interpenetrating network, wherein the interpenetrating network is prepared by synthesizing a first polymer in a matrix of a second polymer solution.

REMARKS

In the Office Action dated December 4, 2002, the Examiner has rejected Claims 3 and 4 under 35 U.S.C. §112 as being indefinite; Claims 1-7 under 35 U.S.C. §102(b) as being anticipated by Dubrow (U.S. Patent No. 5,164,055), Madabhushi et al. (U.S. Patent No. 5,567,292) or Hooper et al. (U.S. Patent No. 5,885,432); and Claims 8-10 have been rejected under 35 U.S.C. §102(b) as anticipated by or, in the alternative, under 35 U.S.C. §103(a) as being unpatentable over Dubrow, Madabhushi et al. or Hooper et al.

Claims 3, 4 and 7 have been amended. New Claims 28 and 29 have been added. Claims 11-27 have been cancelled as being drawn to a nonelected invention. Accordingly, Claims 1-10, 28 and 29 are pending.

Present Invention

A successful separation medium for electrophoresis would include properties such as stability; neutrality; appropriate mesh size; dynamic coating ability (to efficiently suppress electroosmosis); low viscosity, the use of a minimal amount of medium material; and providing good separation results and long read lengths. Before the present invention, a medium that would provide one desirable property, e.g., a long read length, typically would not allow for another desirable property, e.g., dynamic coating ability.

The present invention includes polymer solutions which are useful as media for electrophoresis, in particular, capillary electrophoresis. These polymer solutions comprise a plurality of different polymers in the form of interpenetrating networks (IPNs).

An IPN comprises at least two independent polymer networks which are intermeshed within each other. That is, at least two independent polymer networks are created, penetrate one another, but are not covalently bonded to one another. The IPNs of the present invention do not phase separate during electrophoretic separations.

Each polymer of the IPNs preferably provides different desirable properties to separation media. For example, a poly(acrylamide) (PAM) medium provides good separation results, such as good resolution; a long read length; and a short run time. However, due to its viscosity, the polymers of a PAM medium cannot bind directly to the inner walls of capillary tubes; and therefore, an undesirable electroosmotic flow results during electrophoresis. To avoid this problem, the inner walls of capillary tubes are required to be coated. On the other hand, although polyvinylpyrrolidone

(PVP) does not provide good separation results, PVP is much less viscous and dynamically coats the capillary inner walls. By combining two different polymers in an IPN, e.g., PAM and PVP, the present invention synergistically combines the desirable properties of each polymer into one medium.

Also, by using immiscible polymers in the IPNs of the present invention, the polymer chains stretch out. This stretching out creates a more effective separation medium, and minimizes the amount of polymer material necessary to create the desirable mesh size.

The IPNs of the present invention cannot be prepared by simply dissolving one polymer into another. Instead, for example, a first polymer is formed into a matrix; then, another polymer is polymerized within the matrix.

The IPNs of the present invention have specific structural properties. For example, in the embodiment of Claim 8, the IPNs, formed from a first and second polymer, have a lower weight to volume ratio than the combined weight to volume ratios of the first polymer and the second polymer. (See paragraph bridging pages 14 and 15 in the specification.) Also, as stated in the sentence bridging pages 26 and 27 of the specification, laser light scattering studies evince the formation of interpenetrating networks.

In this amendment, two new claims (claims 29 and 30) have been added. These claims are both in the Jepson claim format. These claims recite methods of separating charged molecular species wherein the improvement is the use of the separation media disclosed in the present invention. Claim 29 describes the separation media in terms of its interpenetrating network structure. Claim 30 describes the separation media in terms of its synthesis.

Rejections under 35 U.S.C. §112

Claims 3 and 4 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

In particular, the Examiner states that in Claim 3, "PVA" "should be enclosed within parentheses so as to avoid confusion with a polyvinylalcohol modified PVA." (Office Action page 2, paragraph 5.) Applicants have amended the claims accordingly.

Additionally, the Examiner has objected to Claim 4 for "contain[ing] the trademark(s)/trade name(s) 'Pluronics' as in EPE-type..." (Office Action page 3, paragraph 6.) Applicants have cancelled "Pluronics" from Claim 4.

Also, the Examiner states that "[t]he term 'P(DMA/DEA)' in Claim 4 is used by the claim to mean 'N,N-diethylacrylamide,' while the accepted meaning is 'a copolymer of N,N-dimethylacrylamide and N,N-diethylacrylamide.'" (Office Action page 3, paragraph 7.)

The Examiner is correct. The term "P(DMA/DEA)" refers to the accepted meaning of a copolymer of N,N-dimethylacrylamide and N,N-diethylacrylamide. See page 5, line 8 of the specification. Applicants have amended Claim 4 to correct this informality.

First Rejection under 35 U.S.C. §102

Claims 1-7 are rejected under 35 U.S.C. §102(b) as being anticipated by Dubrow (U.S. Patent No. 5,164,055).

The Examiner states that “Dubrow teaches a polymer solution containing a **mixture** of water-soluble polymers, exemplified by polyacrylamide and polyethylene oxide, in a network for use in electrophoresic separation.” (Office Action page 4, third paragraph, emphasis added.) The Examiner specifically refers to column 3, lines 50-65 and column 4, lines 40-44 of Dubrow.

The present application discloses **interpenetrating networks** of different polymers. These interpenetrating networks are produced by forming a matrix from a first polymer; and then, polymerizing a second polymer within the matrix. (See page 10, lines 1-2, of the specification.) For example, PAM was synthesized in a PVP solution matrix so that the PAM chains were grown in a network of PVP chains. (See page 26, lines 19-20, of the specification.) Interpenetrating networks cannot be prepared by simply mixing one polymer into another. (See page 26, lines 10-11.)

In contrast, Dubrow does not disclose interpenetrating networks of polymers. Instead, Dubrow describes his matrix as a mixture of a first polymer and a second polymer. (See Claim 2.) Dubrow describes the preparation of his matrices by mixing together two polymers solutions. In particular, Example 6, entitled “Mixed Polymer Matrix,” describes how the Dubrow matrices are made. The two polymers of the matrix are hydroxyethylene cellulose (HEC) and polyacrylamide. The two separate polymer solutions are mixed together along with other ingredients, including a catalyst and a TBE buffer. (See Col. 20, Lines 45-60.)

Also, in the embodiment of Claim 6 of the present application, the interpenetrating network can intrinsically suppress electroosmotic flow (i.e. electrophoendoosmotic flow) simply by the presence of specific polymers within the network itself.

In contrast, Dubrow describes only four possible methods by which to reduce electroosmotic flow in the charged-wall capillary. None of the methods described in Dubrow suppress this flow as embodied in Claim 6 of the present application. In particular, Dubrow states:

If desired, electroosmotic flow in a charged-wall capillary can be substantially eliminated by one of four approaches. In the first approach, the viscosity of the polymer is made sufficiently high...In a second approach, the capillary tube is provided with a water-permeable plug...Another approach is based on chemical bonding of the matrix, after injection into the tube, with the walls of the tube. Here the tube is first chemically treated with a bifunctional coupling agent capable of reacting with chemical groups on the tube wall and with the polymer in the matrix. ... Finally, the electrophoresis tube may have coated walls, such as Teflon-coated walls, to mask surface wall charges or the tube itself may be formed from non-charged polymer material such as Teflon. (See Col. 10, Lines 25-49. Emphasis added.)

Thus, unlike the present invention, Dubrow does not describe a separation medium which can intrinsically suppress the electroosmotic flow.

Since Dubrow does not disclose all the claim limitations of independent Claim 1, namely interpenetrating networks formed by different polymers, Dubrow cannot anticipate the present invention.

Second Rejection under 35 U.S.C. §102

Claims 1-7 are rejected under 35 U.S.C. §102(b) as being anticipated by Madabhushi et al. (U.S. Patent No. 5,567,292).

The Examiner states that “Madabhushi et al. teach a polymer solution containing a **mixture** of water-soluble polymers, at least one representing a ‘silica-absorbing polymer’ of the present claim 7, in a network for use in electrophoresic separation.” (Office Action page 4, fourth paragraph, emphasis added.) The Examiner specifically refers to column 2, lines 34-35; column 5, lines 20-23; examples 7 and 8; and Figure 5 of Madabhushi et al.

The present application discloses **interpenetrating networks** of different polymers. These interpenetrating networks are produced by forming a matrix from a first polymer; and then, polymerizing a second polymer within the matrix. (See page 10, lines 1-2, of the specification.) For example, PAM was synthesized in a PVP solution matrix so that the PAM chains were grown in a network of PVP chains. (See page 26, lines 19-20, of the specification.) Interpenetrating networks cannot be prepared by simply mixing one polymer into another. (See page 26, lines 10-11.)

In contrast, Madabhushi et al. disclose media for separating biomolecules which include a polymeric “sieving component” and a polymeric “surface interaction component.” These media are formed by **mixing** the two components together. (For example, see Col. 13, Lines 1-4.) Accordingly, unlike Claim 1 of the present application, Madabhushi et al. do not disclose interpenetrating networks.

Since Madabhushi et al. do not disclose all the claim limitations of independent Claim 1, namely interpenetrating network, Madabhushi et al. cannot anticipate the present invention.

Third Rejection under 35 U.S.C. §102

Claims 1-7 are rejected under 35 U.S.C. §102(b) as being anticipated by Hooper et al. (U.S. Patent No. 5,885,432).

The Examiner states that “Hooper et al. teach a polymer solution containing a **mixture** of water-soluble polymers, at least one representing a ‘silica-absorbing polymers’ of claim 7, in a network for use in electrophoresic separation.” (Office Action page 4, fifth paragraph, emphasis added.) The Examiner specifically refers to column 2, lines 61-67; column 4, lines 8-67; column 6, lines 5-32; and examples 4, 5, 7 and 8 of Hooper et al.

Hooper et al. disclose an electrophoretic medium which changes its viscosity in response to temperature changes. In order to effect these viscosity changes, this medium includes “un-crosslinked high molecular weight polymers.” (See Col. 3, Lines 50-53.)

In contrast, the specification and the claims of the present application do **not** describe media which change their viscosities in response to temperature changes.

Also, the Examiner states that Hooper et al. teach a “mixture.” As described above, the present application discloses **interpenetrating networks** of different polymers. Interpenetrating networks cannot be prepared by simply mixing one polymer into another.

Since Hooper et al. do not disclose all the claim limitations of independent Claim 1, namely interpenetrating networks, Hooper et al. do not anticipate the present invention.

Rejection under 35 U.S.C. §102/35 U.S.C. §103(a)

Claims 8-10 have been rejected under 35 U.S.C. §102(b) as anticipated by or, in the alternative, under 35 U.S.C. §103(a) as being unpatentable over, Dubrow, Madabhushi et al. or Hooper et al. (Office Action, page 5, paragraph 11).

The Examiner concedes that “Dubrow, Madabhushi et al. or Hooper et al. do not expressly teach the disclosed entanglement properties and base read lengths” of the present invention. The Examiner further states that “it is reasonable that said properties in the polymer solutions of Dubrow, Madabhushi et al. or Hooper et al. would be the same as in the presently claimed solution since the **composition of** Dubrow, Madabhushi et al. or Hooper et al. are essentially the same as the claimed solution.” (Office Action page 5, third paragraph, emphasis added.)

As described above, the present application discloses electrophoretic media comprising polymers in the form of **interpenetrating networks**. As stated by the Examiner, the “entanglement properties” of the present invention are not disclosed by the cited references. The Examiner attempts to remedy this deficiency in the references by stating that the references disclose solutions that contain the same composition as the solutions of the present invention; and thus the solutions of the cited references and the present invention are “essentially the same.”

However, as described above, **in order to produce media comprising the interpenetrating networks of the present invention, the media must be produced in a specific manner as described in the specification.** The Examiner states that “Dubrow, Madabhushi et al. or Hooper et al. do not expressly teach the polymer solutions to be prepared by synthesizing one polymer in the presence of the other...”

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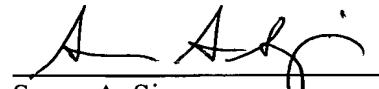
as described by the present invention. (Office Action page 5, last paragraph.)

Thus even if the cited references did disclose solutions that contain the same composition as the solutions of the present invention (for argument's sake only), the media would be different. That is, the media of the prior art and the media of the present invention would be structurally different.

The cited prior art references do not disclose an interpenetrating polymeric structure. Thus, the cited references do not teach or suggest all the limitations recited in the claims. Accordingly, the present invention is not anticipated by, nor obvious over, the cited references.

Applicants respectfully submit that the application is now in condition for allowance, which action is earnestly solicited. If resolution of any remaining issue is required prior to allowance of this application, it is respectfully requested that the Examiner contact Applicants' undersigned attorney at the telephone number provided below. A petition for a two-month extension of time accompanies this amendment.

Respectfully submitted,



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VERSION OF CLAIMS WITH
MARKINGS TO SHOW CHANGE MADE

IN THE CLAIMS:

Please cancel Claims 11-27.

Please amend Claims 3, 4 and 7.

3. (Amended) A polymer solution in accordance with Claim 1, wherein at least one of said polymers is polyacrylamide (“PAM”), N-substituted PAM, N,N-disubstituted PAM, modified polysaccharides, polyethylene oxide (“PEO”), polyvinylpyrrolidone (“PVP”), polyvinylalcohol (“PVA”), polyethylene glycol (“PEG”), or a random, a graft or a block copolymer based on the backbone monomer segments thereof, wherein nitrogen substitutes are selected from the group consisting of C₁ to C₃ alkyl, hydroxyl-substituted C₁ to C₃ alkyl, and methoxy-substituted C₁ to C₃ alkyl.

4. (Amended) A polymer solution in accordance with Claim 3, wherein said random, graft or block copolymer is EPE-type Pluronics, N,N-dimethylacrylamide and N,N-diethylacrylamide (“P(DMA/DEA”), a copolymer of poly(N-isopropylacrylamide) densely grafted with short poly(ethylene oxide) (“PNIPAM-g-PEO”) or polyacrylamide-co-allyl-β-D-glucopyranoside (“P(AM/AG”).

7. (Amended) A polymer solution in accordance with Claim 6, wherein said silica-absorbing polymer is selected from the group consisting of PVP, PEO, EPE-type Pluronics, N-substituted PAM and N,N-disubstituted PAM, and wherein nitrogen substitutes are selected from the group consisting of C₁ to C₃ alkyl, hydroxyl-substituted C₁ to C₃ alkyl, and methoxy-substituted C₁ to C₃ alkyl.

Please add new Claims 28-29.

28. (New) In a method of separating charged molecular species, the method comprising causing a charged molecular species to migrate in a separation medium by the influence of an applied electric field, the improvement wherein the separation medium comprises a plurality of different polymers that do not phase separate when dissolved in solution and that are entangled to form an interpenetrating network.

29. (New) In a method of separating charged molecular species, the method comprising causing a charged molecular species to migrate in a separation medium by the influence of an applied electric field, the improvement wherein the separation medium comprises two different polymers that do not phase separate when dissolved in solution and that are entangled to form an interpenetrating network, wherein the interpenetrating network is prepared by synthesizing a first polymer in a matrix of a second polymer solution.